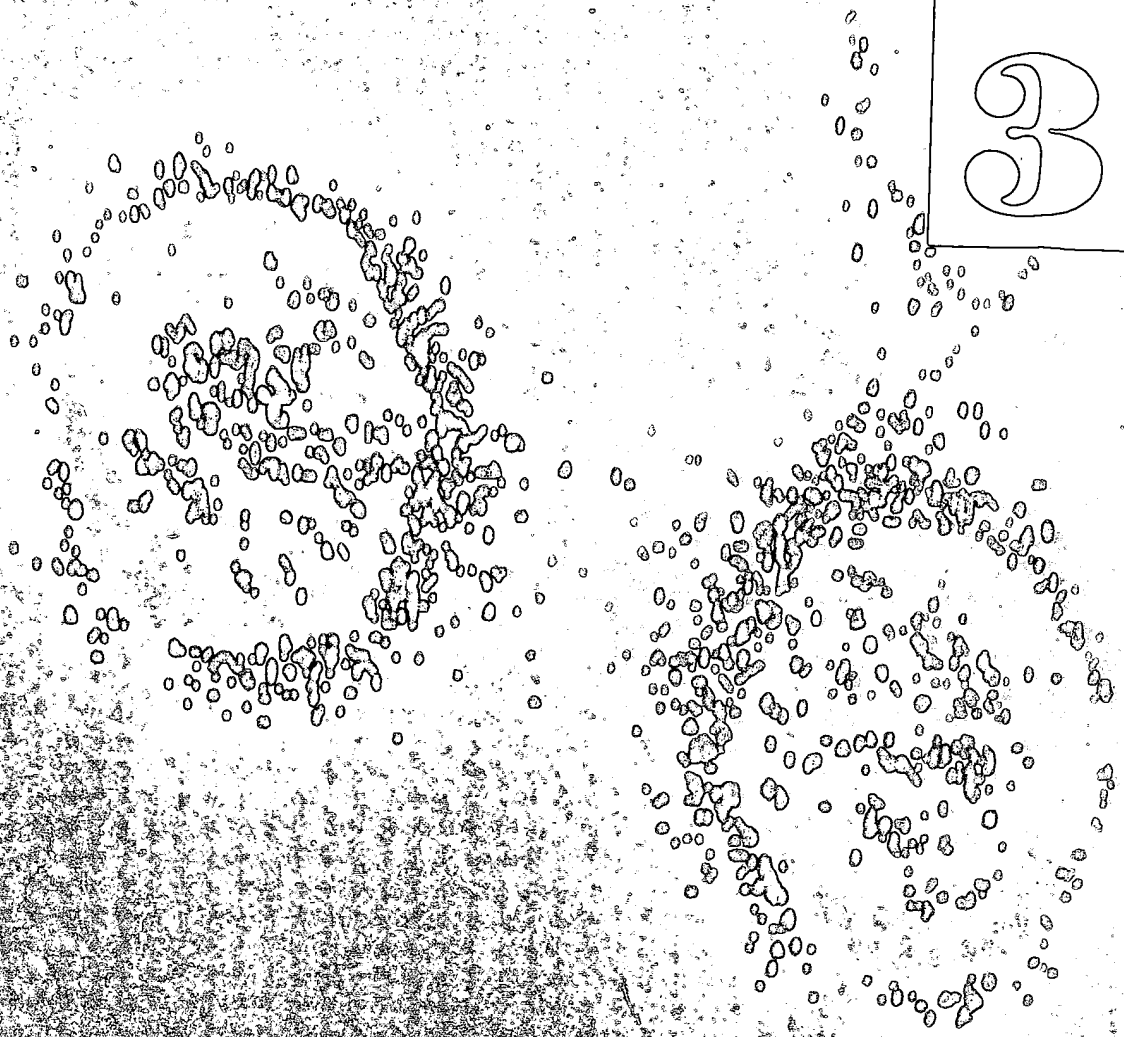

3

Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

Sambrook · Fritsch · Maniatis

Exhibit A

3

Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

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The process of enzymatic conversion of poly(A)$^+$ mRNA to double-stranded DNA and the insertion of this DNA into prokaryotic vectors has become a fundamental tool of eukaryotic molecular biology. Since the first clones of complementary DNA (cDNA) were obtained in the mid-1970s, many different methods have been developed to increase the efficiency of synthesis of double-stranded cDNA and many improvements have been made to the vector systems. In the first part of this chapter, we describe some of these developments, pointing out their advantages and disadvantages. In the second part, we present in detail the procedure that is now most commonly used: synthesis of the first strand of cDNA with reverse transcriptase, replacement synthesis of the second strand of cDNA (using RNAase H and the Klenow fragment of *Escherichia coli* DNA polymerase I), addition of synthetic linkers, and finally, cloning into a bacteriophage λ vector.

The development of methods for the introduction of DNA into cultured mammalian cells has made it possible to express cloned genes in a broad range of cell types from different species. These methods have been used to overproduce proteins for structural and biochemical studies and to identify elements involved in the control of gene expression. In both types of studies, the cloned sequence of interest is inserted into the appropriate expression vector, cloned in bacteria, amplified by replication, and then used to transfect mammalian cells.

In this chapter, we describe a number of commonly used mammalian expression vectors, and we provide protocols for introducing cloned genes into mammalian cells. We begin by discussing expression of proteins and then go on to describe methods used to study gene regulation. In general, different vectors are required for the two types of studies, but many of the basic components used in the construction of these vectors are the same.